PHYSIOLOGICAL PROPERTIES OF BOG WATER.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. LXXII.

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(WITH THREE FIGURES)

ALTHOUGH it has been shown (1) that water from the bogs of the northern United States contains solutes to such a very small amount that its osmotic pressure is generally not appreciably above that of the river swamps and lakes of the same region, still the generally observed xerophilous character of bog vegetation may be due to small amounts of dissolved substances of such nature that they affect the plants chemically through toxic stimulation. Having found that metallic ions affect the vegetative growth of the polymorphic Stigeoclonium with which the author has been experimenting for some time (2), and that the effect thus produced is identical with the response of the alga to high osmotic pressures, it was suggested that this alga might be used as an indicator in a study of the physiological properties of bog waters. In accordance with this suggestion, natural waters of a number of different types were collected in bottles, filtered through filter paper, and tested as culture media for the alga. The result of these tests is, briefly, that many bog waters act upon the plant like poisoned solutions. Details of the work are given in the following pages.

The form of Stigeoclonium here used has already been shown (2–6) to take either of two forms according to the medium in which it is grown. In solutions of low osmotic pressure at ordinary temperatures it assumes the form of branching filaments composed of cylindrical cells. In the same solutions at a temperature slightly above the freezing point of water, and at ordinary temperatures in solutions similar to these but poisoned with certain metallic salts, as well as at ordinary temperatures in solutions of high osmotic pressure, the plant takes the palmella form, in which the cells are spherical or nearly so and lie in the medium singly or in irregular groups. If filaments are taken from the conditions which favor their growth and are placed in those favoring the palmella form, their cells round off and partially or completely separate, they begin to divide slowly in all planes, and the result is the typical form for these conditions. A return to the conditions for filaments is followed by the resumption of that form, partly by the growth of filaments directly from the palmella masses, and partly by the production of zoospores which germinate to form new filaments. Zoospores are not produced in solutions of high pressure, and they fail to germinate in solutions which produce the palmella form, though they are produced in the cold and in poisoned solutions.

The waters tested in these experiments are in large part the same as were used in the determination of the osmotic pressure (1). The work was carried on partly at the New York Botanical Garden and partly at the University of Chicago, and extended from June 1902 to January 1905. The cultures were made in the manner described for water cultures of this plant in the author's earlier papers.

Since a number of authors have loosely attributed SCHIMPER'S "physiological dryness" (7) of bogs to acidity, titrations of most of the natural waters here employed were made with *n*/100 KOH solution, using phenolphthalein as indicator.[1]

Data from the experiments are given in the following table. In the column of responses, F—F denotes that the filamentous form persisted as such when placed in this particular water. F—P denotes that the original filaments became palmella and that no new filaments were produced. F—½P denotes that filaments persisted, but that there was also a marked production of palmella. A number of cultures were made with each of the waters, and the result is the general one for all. The cultures were continued for two to four weeks. The acidity data are given in terms of normal acid and the pressure in terms of millimeters of mercury at 25° C.

From the table it is seen at once that in some of the waters the palmella form was produced; in others it was produced in some measure, but filaments persisted; and in still others the filamentous

[1] Of the indicators at hand this was the best, although it is assuredly not perfect for such acids as are probably present in these waters.

DATA FROM THE EXPERIMENTS

Source of water and nature of vegetation	Response of Stigeo-clonium	Osmotic pressure of water, mm. of Hg	Acidity of water in terms of normal acid
Drained swamps of Hackensack River, N. J. (river swamps)—			
Average of six samples	F—½P	50	0.00192
Maximum of six samples	.....	..	0.0042
Minimum of six samples	.....	..	0.001
New York City supply, Croton and Bronx Rivers	F—F	50	0.0005
Chicago City supply, Lake Michigan	F—F (see *fig. 1*)	100	Always alkaline, about 0.0015
Grand River, Grand Rapids, Mich	F—F	100	As last, or more alkaline
Aetna, Ind. (Rhus vernix, Drosera)—			
Average of three samples pressed from black peat	F—½P	100	......
Miller, Ind. (Larix, Rhus vernix)—			
Average of two samples pressed from black peat	F—½P	50	0.0003
Oconomowoc, Wis.* (typical Larix swamp), ditch	F—P	200	0.0004
Stewart Ridge, Ill., average of three samples pressed from black peat	F—F	150	0.0002
Ann Arbor, Mich.†			
First Sister Lake bog (Larix, Chamaedaphne, Sphagnum)—			
Sample A	F—P (see *fig. 2*)	50	0.0033
Sample B	F—P	440	0.0038
West Lake bog (Sphagnum, Chamaedaphne, Potentilla palustris, Salix)—			
Sample A	F—P	100	0.0026
Sample B	F—P (see *fig. 3*)	150	0.0024
Tom's River, N. J. (Chamaecyparis, Sphagnum, Chamaedaphne, Sarracenia, Oxycoccus)—			
Sample A, pressed from Sphagnum	F—F	170	0.0004
Sample B, beneath Sphagnum	F—P	140	0.00048
Sample C, pressed from black peat	F—P	90	0.0003
Sample D, margin of pond	F—P	40	0.0003
Sample E, ditch	F—P	50	0.0003
Richmond, Staten Island, N. Y. (Alnus, Eriophorum, Sphagnum)			
Sample A, edge of pond	F—P	90	0.0015
Sample B, decayed leaves	F—½P	110	0.0022
Sample C, ditch	F—F	90	0.0008
Sample D, pressed from Sphagnum	F—F	100	0.001

* This water was obtained for me by Dr. H. C. COWLES of this laboratory.

† The samples from Ann Arbor were obtained for me by Dr. H. N. TRANSEAU, of Alma College, Mich.

form persisted without the production of palmella at all. The characteristic forms of this plant in three different waters are shown in *figs. 1, 2,* and *3,* which are to be compared with previously published figures, and are self-explanatory.

As has been shown in the case of many inorganic poisons (5), the production of the palmella form is sometimes accompanied in these waters by a stimulation of zoospore production. Usually, however, the swamp water acts more like low temperatures, producing the vegetative response without either accelerating or retarding the reproductive activity.

The palmella response in certain of these waters may be due, *a priori*, to either of two sets of factors, the osmotic pressure of the solution or the chemical nature of the solutes. Since the experiments were carried on at room temperature, it is unnecessary to consider low temperature as a possible stimulus.

In the work on the influence of osmotic pressure upon this plant it was found (2, 3) that there is no tendency to form palmella till a pressure of about 1618.6^{mm} of mercury has been attained. Filaments still persist at a pressure of 3237.1^{mm}, but have practically all disappeared at a pressure of 6474.2^{mm}. But no swamp water studied has a pressure at all approaching the lower limit for even the incipient

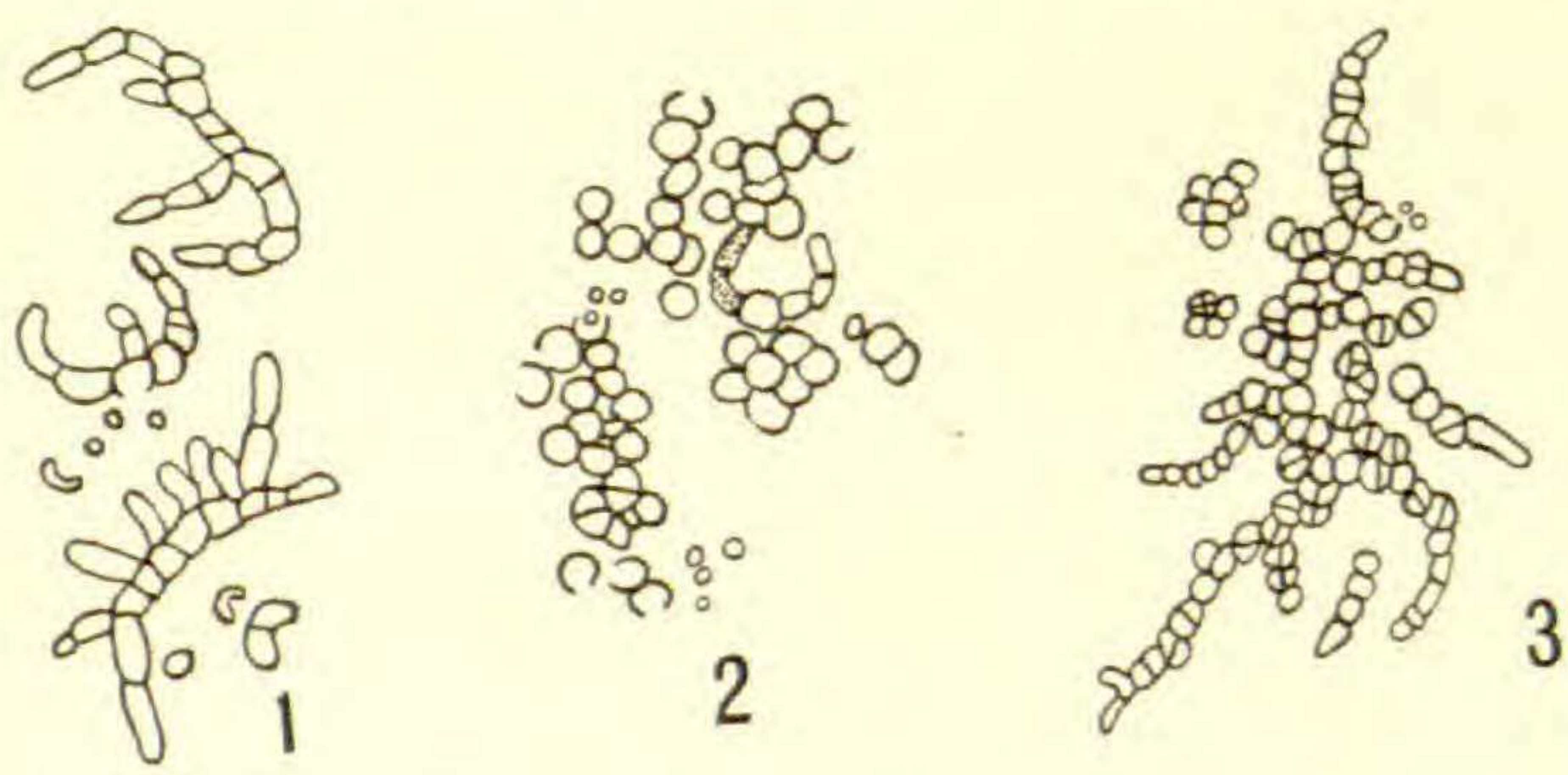

FIG. 1. Stigeoclonium filaments in Lake Michigan water.

FIG. 2. Stigeoclonium, palmella form from filaments, in water from West Lake, Ann Arbor, Michigan, sample A.

FIG. 3. As *fig. 2*, but in water from First Sister Lake, Ann Arbor, sample B.

formation of palmella. Therefore, we are forced to the conclusion that the palmella response in these bog waters is due to the chemical nature of the solutes. The plant grows well as filaments for a long time in distilled water, so that it is impossible to relate the reasons to absence of inorganic salts.

Definite knowledge of the chemistry of bog water is beyond our reach at present. It appears that all bogs are acid to some degree, and there has been a tendency for many authors, *e. g.*, SCHIMPER (7, pp. 4, 8, 657, etc.), to attribute the peculiarities of bog plants to this property. In the author's study of chemical stimulation (5) it was found that nitric and sulfuric acids produce palmella at concentrations of from $0.0001n$ to $0.00006n$. The natural swamp waters are uniformly more acid than this; therefore, were the acid property of the latter due to either of these mineral acids, we should expect *all* of these waters to produce the palmella response. This is obviously not the case, and so it seems highly probable at least that the stimulating factor of bog waters is not the hydrogen ion.

Further, a comparison of the acidity figures with the response of the plant in the different waters shows clearly that the former data could not be used as a criterion for the prediction of the latter. This is clearly brought out in the following list, in which the acidity figures are arranged in order of their magnitude, with the responses occurring in the corresponding water placed after each. Alkalinity 0.0015*n*, F; acidity 0.0002*n*, F; 0.0003*n*, F, ½P, P; 0.0004*n*, F, P; 0.00048*n*, P; 0.0005*n*, F; 0.0008*n*, F; 0.001*n*, F; 0.0015*n*, P; 0.0019*n*, ½P; 0.0022*n*, ½P, P; 0.0026*n*, P; 0.0033*n*, P; 0.0038*n*, P. The lower acidities appear to produce both filaments and palmella, the higher ones only palmella. This would seem to indicate that, while high acidity is always accompanied by the presence of the stimulating substances, these substances are not necessarily accompanied by high acidity.

Boiling the stimulating waters for five or ten minutes and then rediluting to the original volume with redistilled water decreases their acidity from 30 to 50 per cent., but appears not to alter their stimulating power. Apparently the active substances are not volatile at 100° C. Diluting the Ann Arbor samples, and also those from Tom's River numbered *2*, *3*, and *4*, with distilled water or with weak nutrient solution, decreases the toxic effect, and this effect practically disappears when the water has been diluted to twice its volume. This is evidence that the stimulating substances are present in extremely small amount.

The relation of the source of these waters and the type of vegetation growing therein to the physiological properties exhibited toward Stigeoclonium throws some light on the general problem of the xerophilous character of bog plants. The drained swamps of the Hackensack valley are not in any sense bogs. In many places, however, are found spots where Sphagnum has taken a foothold in small pools. Eriophorum, Typha, and some other semi-xerophilous plants are also found here. The water samples studied were taken from such places, and the experiments show that they possess the toxic property to a considerable degree. The data for this broad area of swamps are averaged from a large number of samples taken near Englewood and Closter, N. J., as well as from the western slope above the palisades of the Hudson, where the water is held in the irregular rock basins.

The New York City water, although quite acid, is not active upon the alga. This water is from streams with well-drained river swamp margins. Lake Michigan water and the water of Grand River appear to be identical. Both are somewhat alkaline; neither has any physiological effect upon Stigeoclonium.

The Aetna and Miller swamps are practically alike; both are composed of black peat with no Sphagnum. The general nature of these swamps suggests an intermediate condition between bog and river swamp, leaning toward the former, and the water shows a marked tendency to produce palmella.

The bog near Oconomowoc has Larix, Sphagnum, Vaccinium corymbosum, Oxycoccus, Chamaedaphne, Sarracenia, etc. Its water has a very marked action upon the alga.

The Stewart Ridge swamp is a peat deposit but not a true bog. A few patches of Sphagnum seem to show a tendency in this direction, but the samples tested showed no effect upon the indicator plant.

The swamps of Ann Arbor are as typical and characteristic bogs as the author has seen. The character of their vegetation, consisting of Larix, Drosera, Sarracenia, Andromeda, Chamaedaphne, Arethusa, Calopogon, etc., agrees well with the fact that the water is markedly toxic toward the alga.

The vegetation upon all the vast stretches of lowland about Tom's River is practically alike; these are dense Chamaecyparis swamps, abounding in Oxycoccus, Sphagnum, Sarracenia, Chamaedaphne, etc. All the samples from here, with the exception of one pressed from living moss, produced the palmella response. It appears that the active bodies are more plentiful in the mass of dead material beneath the Sphagnum than in the moss itself.

The Richmond swamp is not a true bog, and yet it contains considerable amounts of loosely-growing Sphagnum, together with Eriophorum, Typha, etc. Here there appears a disagreement between the physiological properties of water samples from different parts. That from the ditch should be the most dilute, and shows no action upon the indicator. That from moss is again harmless; while that pressed from decayed leaves near by shows a marked toxic effect. The pond sample was taken up within a centimeter or two of submerged powdery peat, and should be saturated or nearly so with any slightly soluble substances contained therein. Its active property is very marked.

From the last eight paragraphs it seems clear that the stimulating substances with which we have been dealing are present in swamp waters to an extent roughly proportional to the xerophilous character exhibited by the swamp vegetation. It is possible that the factor in such bogs which prevents the growth of plants other than xerophilous ones may be these unknown toxic bodies. They act upon Stigeoclonium in much the same manner as do drying media. Perhaps ordinary plants are affected by these substances with the same end result as though they were in a truly dry soil. If this be true it becomes easy to see how plants whose protoplasm is naturally adapted to dry situations may alone be able to thrive in these bogs.

The behavior of this alga toward dryness, cold, and bog water are quite parallel with results obtained by TRANSEAU (8) with *Rumex acetosella*. This author found that in dry mineral soil, Rumex produces thickened leaves reduced in size and with revolute margins, while the palisade tissue is very much increased in amount, and the epidermal cells are reduced in size and have thick, cuticularized outer walls. These changes give the plant, which in moist conditions is anything but a xerophyte, a very characteristic xerophilous structure. The same responses are exhibited, to a somewhat less degree, when the roots are kept at a low temperature, and also when the substratum is a bog soil. But when both the last named conditions are allowed to act together the response is the same in direction and amount as in dry mineral soils. The changes occurring in these leaves are very similar in their nature to those just described for Stigeoclonium. From an ecological standpoint, the palmella form of the alga is extremely xerophilous in character, while the filamentous form lies at the other extreme. It appears that we have here two very widely different plants, both of which respond to these various conditions in the same way.

SUMMARY.

The results of this series of experiments are as follows:

1. There are chemical substances, in at least some bog waters, which affect Stigeoclonium as do poisoned solutions and solutions of high osmotic pressure.

2. The responses of this alga to bog water and to cold are as nearly identical with those obtained by TRANSEAU with Rumex, for the same conditions, as the nature of the two plants would permit.

3. The active substances are not directly related to the acidity of the water.

4. Boiling the water decreases its acidity but does not appreciably affect its action as a stimulating agent.

5. The stimulating substances are most markedly present in water from those swamps whose vegetation is most definitely of the bog type. They are absent from river swamps and large lakes; in water from swamps whose vegetation is of a character intermediate between those of the river swamp and the bog, they are present to some degree, their amount being roughly proportional to the extent of the xerophilous character of the vegetation.

6. The stimulating substances here demonstrated may play an important rôle in the inhibition from bogs of plants other than those of xerophilous habit.

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